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Reflection of research on auditory brainstem responses of normal and mutant mice

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Reflection of Research on Auditory Brainstem Responses of Normal and Mutant Mice

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A Project Presented to

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College of Health and Behavioral Studies

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by Valerie Yasamin Acquesta

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Accepted by the faculty of the Department of Communication Sciences & Disorders, James Madison University, in partial fulfillment of the requirements for the Degree of Bachelor of Arts

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Acknowledgments

I can't express enough thanks to my committee for their support and encouragement throughout this process. Thanks to Dr. Chris Clinard and Dr. Mark Gabriele. I sincerely appreciative and am grateful for the support of my advisor, Dr. Lincoln Gray, whose dedication and love for research is inspiring. I also thank Will Noftz who was a great partner and mentor.

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Reflection

For the last year I have been working with Will Noftz, a James Madison University biology graduate student, on collecting data for his dissertation. His research focuses on the developing auditory system of mice. The research focused on the involvement of Eph receptors and their ligands on the development of tonotopic maps and patterns in the auditory brainstem. Specifically, his research focused on ephrin-B3, the protein encoded by the EFNB3 gene (Tuzi and Gullick, 1994). I aided Will in collecting data on the auditory brainstem responses (ABR) of mice deficient in ephrin-B3 (ephrin-B3^{null}), and mice with the disruption of reverse signaling (ephrin-B3^{lacZ}), and wild-type mice. We analyzed the functions of ABR latency-intensity function in the early auditory system up to the inferior colliculus (IC). Comparison of the control group, wild-type mice, revealed the effects of ephrin-B3 on the development of the auditory system.

Beginning this research I had expectations of gaining a better understanding of the auditory system and enhancing my skills in the laboratory. My experience exceeded my expectations. This research opportunity has impacted me personally and academically. On a personal level, this research experience has greatly formed my future and my career path. Through this process, I decided to change my future scope of study. I began building upon my existing knowledge of the auditory system, and realized how I could direct it towards helping others. I have also gained valuable academic experience. I obtained a better understanding of the work behind research. I acquired methodology and concepts that a classroom

setting could not have provided, such as techniques for performing ABRs. This research opportunity has also shown me the value of being meticulous in my preparation and work. I learned how to balance individual work and collaborative work. In addition, I learned new troubleshooting skills, such as developing strategies to solve unforeseen problems. For example, these life lessons and skills obtained during my research will greatly affect me academically and personally beyond my college career.

Prior to doing research, I was accustomed to the profound impact hearing has on our daily lives. It empowers us and enriches our lives. It allows us to gather, process, and interpret sounds in our surroundings. Hearing affects our ability to interact in meaningful conversations, and even enjoy aesthetic pleasures like music. Auditory processing gives us the ability to understand auditory information, for example the interpretation of meaningful sounds from background noise. The acoustic perceptions of complex sounds are affected by many factors. A simple pure tone, the simplest of sounds, frequency, intensity, and the timing of sound stimuli. In order to analyze sounds, the auditory system of the receiver has to analyze all three dimensions of sound.

My classes focus exclusively on the auditory system in humans. I was able to take my previous knowledge on the auditory system and funnel it into my research. The functions of sound are determined as the acoustic information travels through the complex auditory pathway. Auditory processing is the transformation and transmission of the auditory signal in the brain after proceeding through the outer,

middle, and inner ear. After proceeding through the middle ear, sound entering the cochlea creates a motion of waves within its fluid spaces. The waves travel varying distances from base to apex of the cochlea, depending on their frequencies displacing the basilar membrane (Dooling, 1989). This acoustic information is received by the auditory receptors in the cochlea. Next, the signal is then transmitted by the auditory nerve, VIII cranial nerve, to the ipsilateral cochlear nucleus in the brainstem. From the ipsilateral cochlear nucleus, the signal is transmitted to the ipsilateral and contralateral superior olive (Dooling, 1989). From the ipsilateral and the contralateral superior olive, the signal ascends in the lateral lemniscus to the IC and the medial geniculate body, until it reaches the auditory cortex in the cerebrum (Dooling, 1989).

The transmission of the auditory signal into sounds we perceive also involves multifactorial structures. Tonotopic maps, which are found throughout the nervous system, maintain the spatial order of neurons in the order of their axonal connections (Tessier-Lavigne, 1995). The IC is organized tonotopically. High frequencies are coded in the ventromedial part of the central nucleus, and low frequencies are coded in the dorsolateral regions (Kelly, Liscum, Van Adel, & Ito, 1998).

I was able to broaden my knowledge of the auditory system by studying the auditory system in mice. The mouse cochlea reaches maturity in eight days, a faster rate than in humans. The absolute auditory threshold curves of mice show an optimum sensitivity between 15 and 20 kHz (Dooling, 1989). A mouse has a

"microtype" middle ear (Fleischer, 1978), meaning it has good transmission of high frequencies due to its thick and narrow basilar membrane (Ehret & Frankenreiter, 1977). This thick and narrow basilar membrane extends the frequency limit of hearing in mice beyond 100 kHz (Dooling, 1989).

In my classes, I've learned that ABRs allow for the analysis of the cochlea and the brain pathways that the auditory information travels. The evoked potentials are generated by a brief click or tone pip transmitted from an acoustic transducer. In my classes, visualizing this process was very vague. During my research, I gained a better understanding of the entire process of ABRs by actively performing ABRs on mice. A mouse's ABR consists of five peaks, which corresponds to waves I-V. The first peak, which represents wave I, is generated by action potential of the auditory nerve after the stimuli was transmitted. Wave II is generated by the ipsilateral cochlear nucleus. Wave III is from the contralateral superior olivary complex. Wave IV is generated bilaterally from the lateral lemniscus, and wave V is generated from the contralateral IC (Melcher & Kiang, 1996). In mice, wave I is the most robust wave, unlike in humans, where wave V is the most robust wave. Wave V in mice is difficult to identify because of the tendency of the noise floor to affect the waveform.

During this research opportunity, I also learned methodology and concepts that were more advanced than those introduced to me in my classes. I learned that the complex auditory pathway consists of mechanisms that form the precise circuitry within the auditory system. I also became familiar with the functions of

Eph receptors contributing to the circuitry within the auditory system. Eph receptor tyrosine kinases (Ephs) and their ligands (ephrins) are a large family of molecules that mediate intercellular signaling (Gale et al., 1996). Eph-ephrins are a family of receptor tyrosine kinases that are membrane-bound proteins. They provide cell-to-cell interactions necessary for a number of physiological and pathological processes (Pasquale, 2008). Eph receptors impact a variety of developmental processes: the role of boundary segmentation, ion transport, axonal guidance, providing cues to axons in the central nervous system, and axonal path-finding (Henkemeyer et al., 1994). These developmental processes depend on the interaction of Eph receptors with the ephrins (Pasquale, 2008).

A distinct feature of Eph-eprins is that they have the ability to generate bidirectional signals (ephrin-to-Eph, *forward*; Eph-to ephrin, *reverse)* (Pickles, Claxton, & Van Heumen, 2002). Eph-ephrins are divided into two subclasses, subclass A and B. In this research we focused on ephrin-B3. Ephrin-B3 is prevalent in the nervous system, where they take a role in establishing neuronal connectivity by guiding axons to their targets (Pasquale, 2008).

It is established that Eph-ephrin interactions play a role in both the development and organization of the auditory system. Eph receptors have numerous roles in the development of the central auditory structure and hearing. "The central auditory projections within the brainstem are topographically organized, reflecting the highly ordered arrangement of best frequencies originating in the cochlea" (Miko, Nakamura, Henkemeyer, & Cramer, 2007). The formation of

these tonotopic projections depends on mechanisms that guide axons. The Eph-ephrin interactions are involved in the development of tonotopic order in the cochlear nucleus and the superior olivary complex (Miko, Nakamura, Henkemeyer, & Cramer, 2007).

I also observed practical techniques for performing ABRs through collaborative work. Throughout the process of this research, I became accustomed to learning from my mistakes and improving upon my procedures with a course of action. This research experience has greatly demonstrated to me the relevance my classes to potential career paths. Engaging in first-hand research allowed me to have a better understanding in the reasoning of research discussed in my classes, as well as developing skills in independent and collaborative work. Before this research opportunity, I shied away from group work. I have always held my studies in high regard, so naturally I would dominate group work. I found it difficult to rely on the work of others. I was continually swamped with work designed for a group of people, because I couldn't rely on my group members. In these situations, my group members generally didn't mind taking a back seat, but during this research I had to take the backseat, a place where I didn't often find myself. I had to work with people who have different personalities than me. I was encouraged to appreciate the different points-of-view of my team, and work toward problem-solving collaboratively. At times we had different views, so I was required to explain my ideas to others in a coherent and convincing manner. I had to support my ideas, and hear rebuttals from my team members. From these rebuttals, I came away

with a new understanding of different ways of thinking. Rather than solving problems on my own, I had to compare my ideas with others and find a compromise that benefited our research.

I took from this opportunity the realization that there are pros to collaborative work. Collaborative work allowed for the presentation of a way of thinking I was unfamiliar with, and allowed for the creation of innovative approaches to projects. I learned to expand my train of thought, and to think outside the box. An example that comes to mind is when Will and I set up the rig for the ABRs. We had to find a constructive way to allow the speaker to be placed into the mouse's ear canal. At first, we had difficulties constructing the rig that was clean-cut and easy-to-use. After brainstorming and deliberating over different ideas, we came up with an innovative solution that increased quality of our research. Our new rig decreased the clutter in the sound booth, and made for an easy-to-use ABR rig. This improved rig reduced the possibility of misplacing the speaker in the mouse's ear canal.

As my mentor Dr. Lincoln Gray says, "perfection is elusive." After performing countless ABRs and testing each mouse twice, in order to acquire accurate waveforms, we collected our waveforms and peak-picked for each wave. We picked peaks for waves I, II, III, IV, and V. Also, we picked troughs for wave I and II. At first, Will and I worked together in a trial session. We collaboratively picked peaks in order to acclimate ourselves with the program and to gain a sense of unison with peak-picking. We then separated and continued to peak-pick on our own. This

individual peak-picking allowed for inter-observable reliability. Unfortunately, after honing our ABR skills while initially working together at peak-picking, our peaks were considerably different when we worked individually.

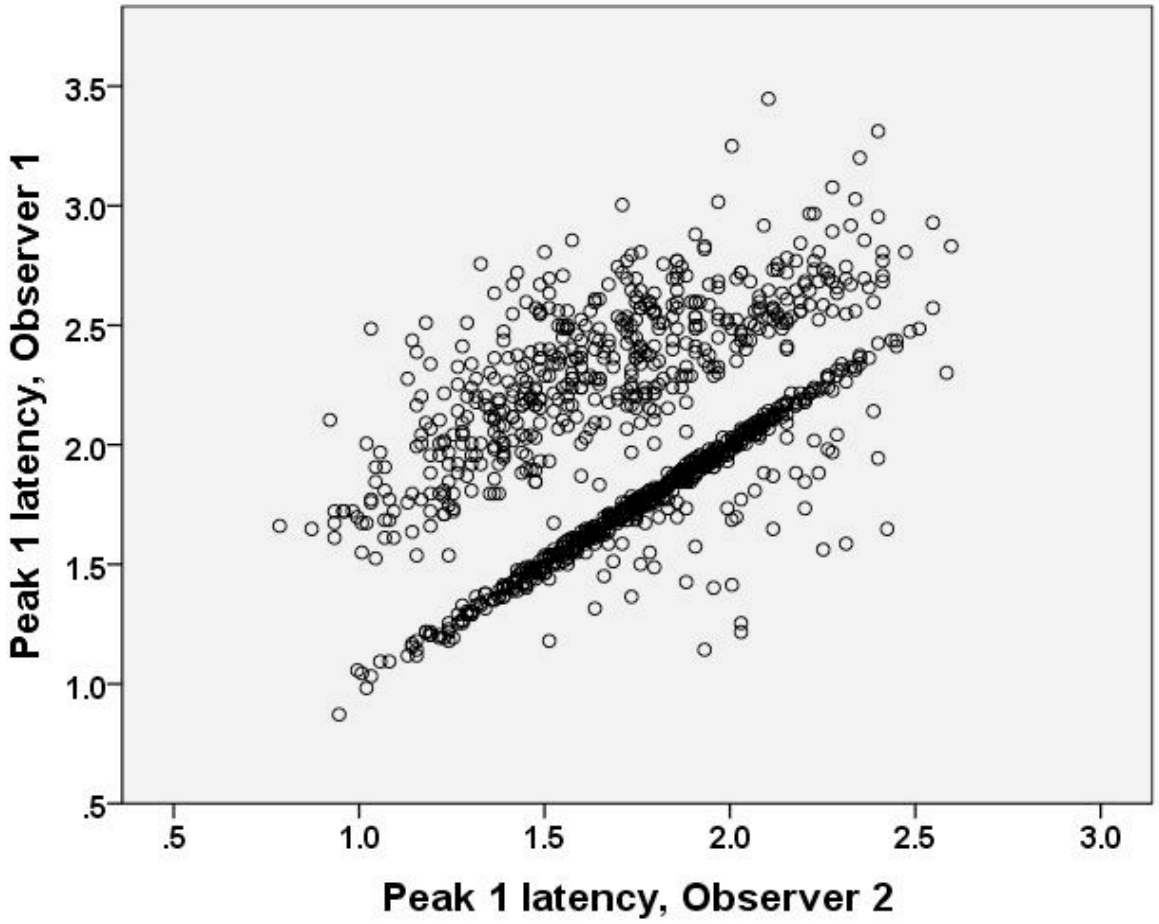

Figure 1. Peak 1 latency of observer 1 and 2

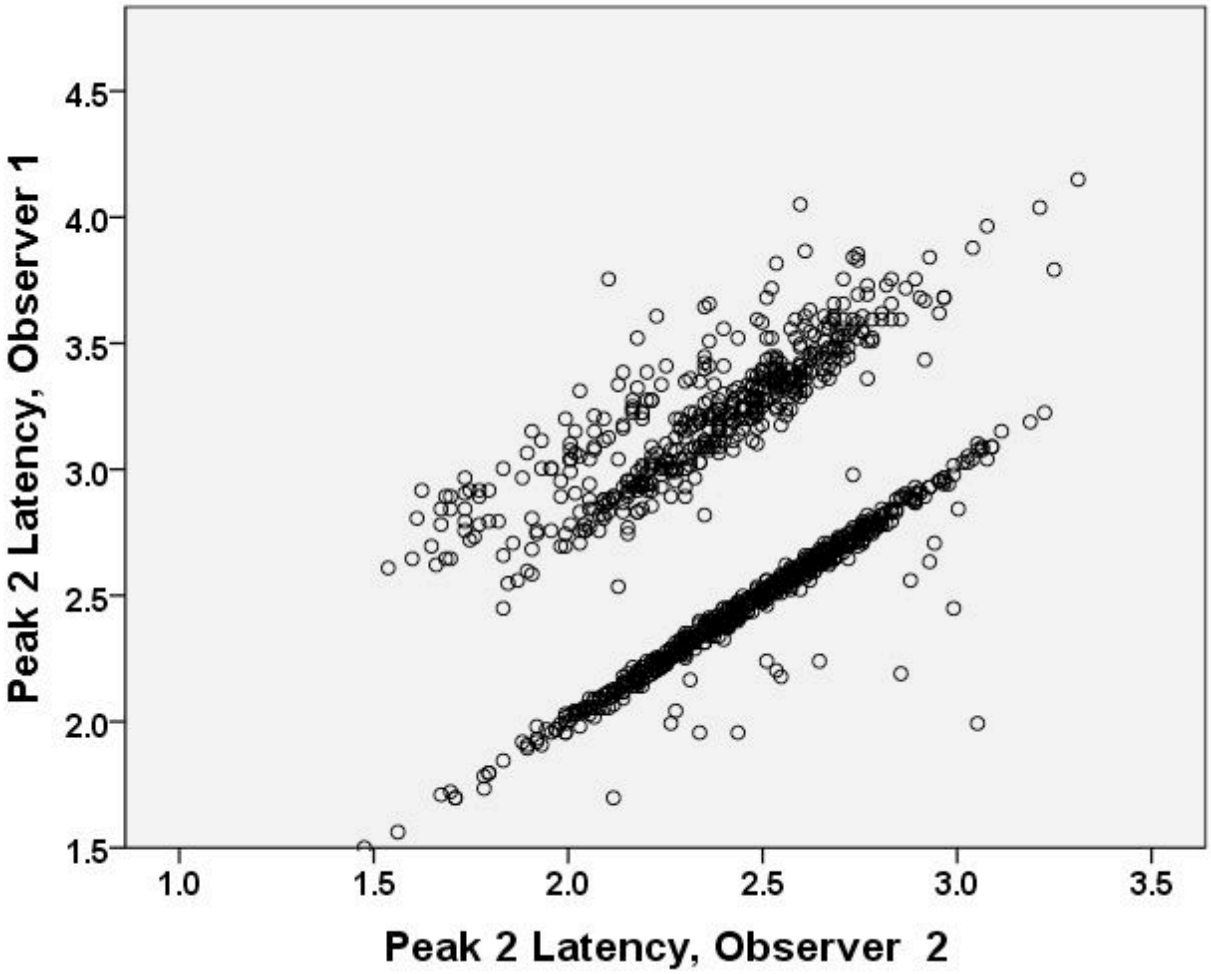

Figure 2. Peak 2 latency of observer 1 and 2

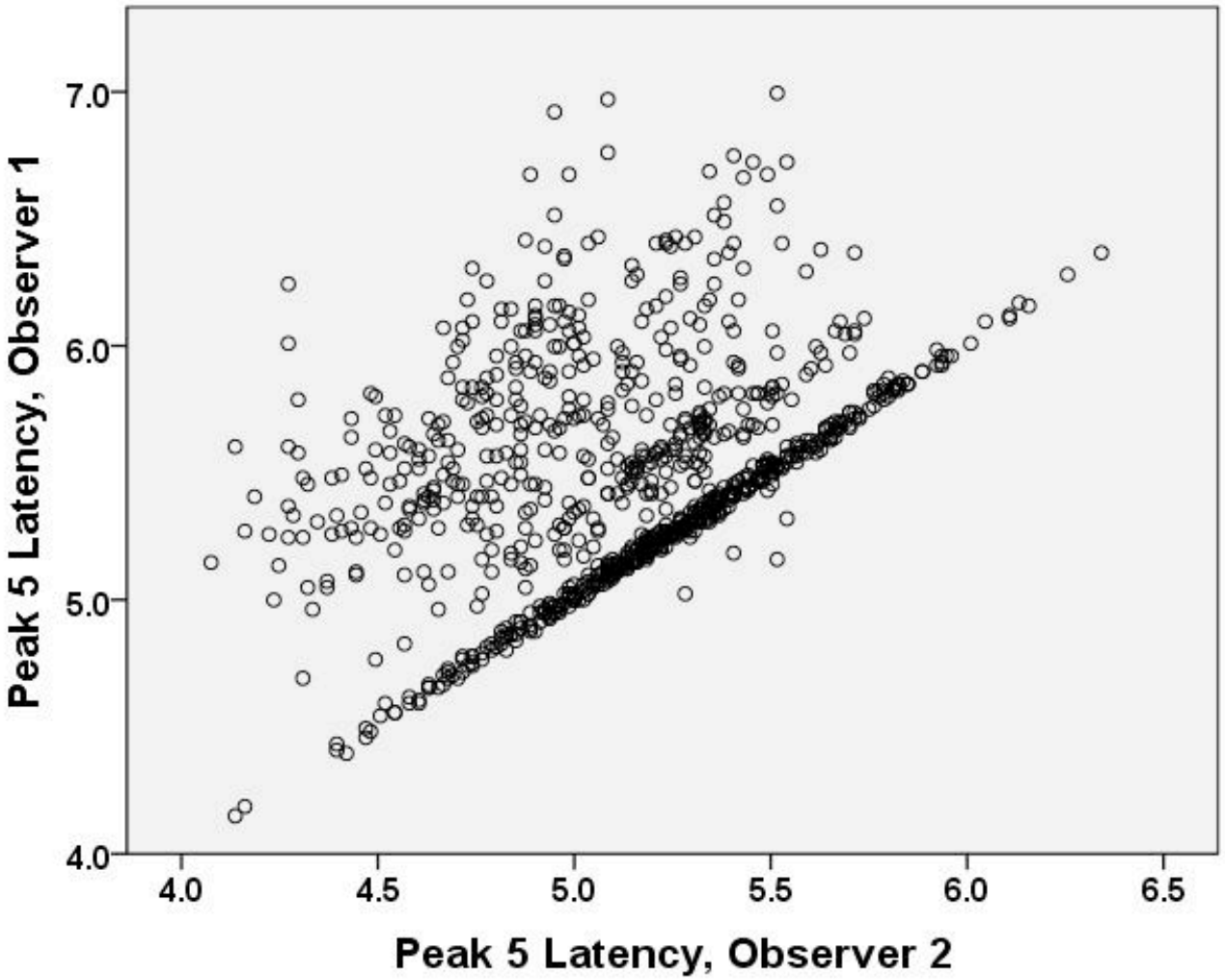

Figure 3. Peak 4 latency of observer 1 and 2

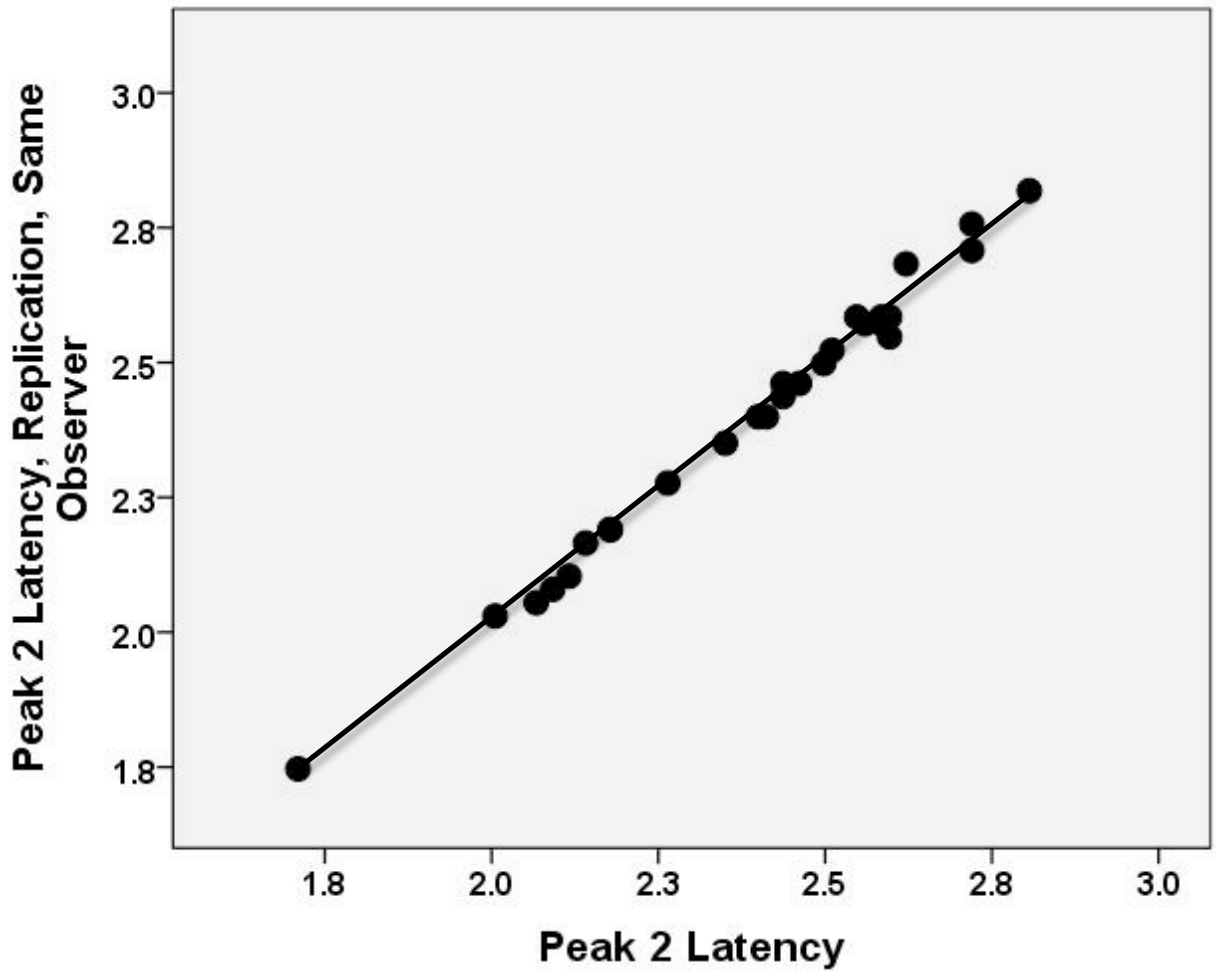

Figure 4. Peak 2 latency of observer 1 primary and replicated peaks

There is a dark region which indicates that observer 1 and observer 2 (Will and I) agreed on peaks, but there is also a considerable amount of outliers (Figure 1, 2, 3). Observer 1 consistently picked latencies for peaks 1, 2, and 5 with a higher latency (Figure 1, 2, 3). In order to qualify the consistency of an observer the ABR waveforms of a few mice were pick-peaked twice. The linear line correlates with a slope of 1, indicating that the observer consistently picked-peaks.

This information was troubling. Our inter-observer reliability illustrated a discrepancy. It was eye-opening that after performing our research to the best of our abilities, the results collected didn't meet our expectations. Through this process, I came to the realization that perfection was actually elusive, and that you can't skew your data to fit your preconceived expectations. You have to take your data as it is. I was able to actively participate in the discussion of possibilities that may have lead to the discrepancies in our inter-observable reliability. Some issues may have been due to peak-picking, the genotypes of the mouse tested, or programming errors. We are continuing to diligently work towards discovering reasons for our discrepant peak-picking, and devising a possible method to improve accuracy, precision, and consistency.

Another revelation I had during this process is that I became very interested in broadening my horizon beyond speech and hearing. As a Communication Sciences & Disorders student, my classes primarily focused on the speech and hearing processes of humans, and disorders that may be related to them. Through my research process, I realized the direct impacts that research could have on an individual. Discoveries found in research can lead to breakthroughs that could benefit someone's life. This thrill of potentially impacting a life fueled my new passion to become a pre-medical student. I wanted to have more of an impact on someone's life and also I wanted to add to my pool of knowledge. So, I declared pre-med and threw myself into a new field of study. In the beginning of my research, I had a very broad understanding of the effects of receptors and their ligands on

hearing. Learning about subjects in my biology classes that were related to my research brought joy to me. I loved learning about biology and its complexities.

The highlight of this research opportunity was furthering my interest in the auditory system. Working alongside colleagues that have the same passion was enlightening and motivating for me in my classes. I knew the auditory system impacted an individual's life in so many ways, and I had a broad understanding behind the mechanisms involved in the amplification and the interpretation of sounds, but I didn't comprehend the aspects of hearing on a molecular level. I believe this research gave me an advantage over my peers. In our classes we were not introduced to the basic molecular level of life. The concepts we learned were very shallow and didn't explore the underlying causes of many of the issues we discussed. In my classes we have discussed genetic mutations that lead to disorders that ultimately lead to a loss of hearing. I continually found myself speculating on the abnormalities in the genome that leads to these complications. But, in our field of study, we didn't question those causes, which left me wanting to acquire a deeper understanding of biology. It was bothersome that our classes didn't introduce basic aspects of biology that I found essential to the study of hearing. Hearing is a universal sense shared between humans, it is a basic life process.

This research opportunity has shaped my future more than I would have ever imagined. Going into this research I wouldn't have dreamed of it having such an impact. I would never have thought I would become a pre-medical student, and be applying to medical schools in the future. Although my last year and a half at JMU

has been a whirlwind of endless classes in order to reach the requirements needed to apply to medical school, I am incredibly thankful I took this opportunity and embraced it to its fullest. I will take from this experience the skills and knowledge I acquired from collaborative work and troubleshooting skills, and hopefully channel my new skills in my future studies and in my personal life. I discovered an entirely new avenue of research, and learned that perfection is elusive.

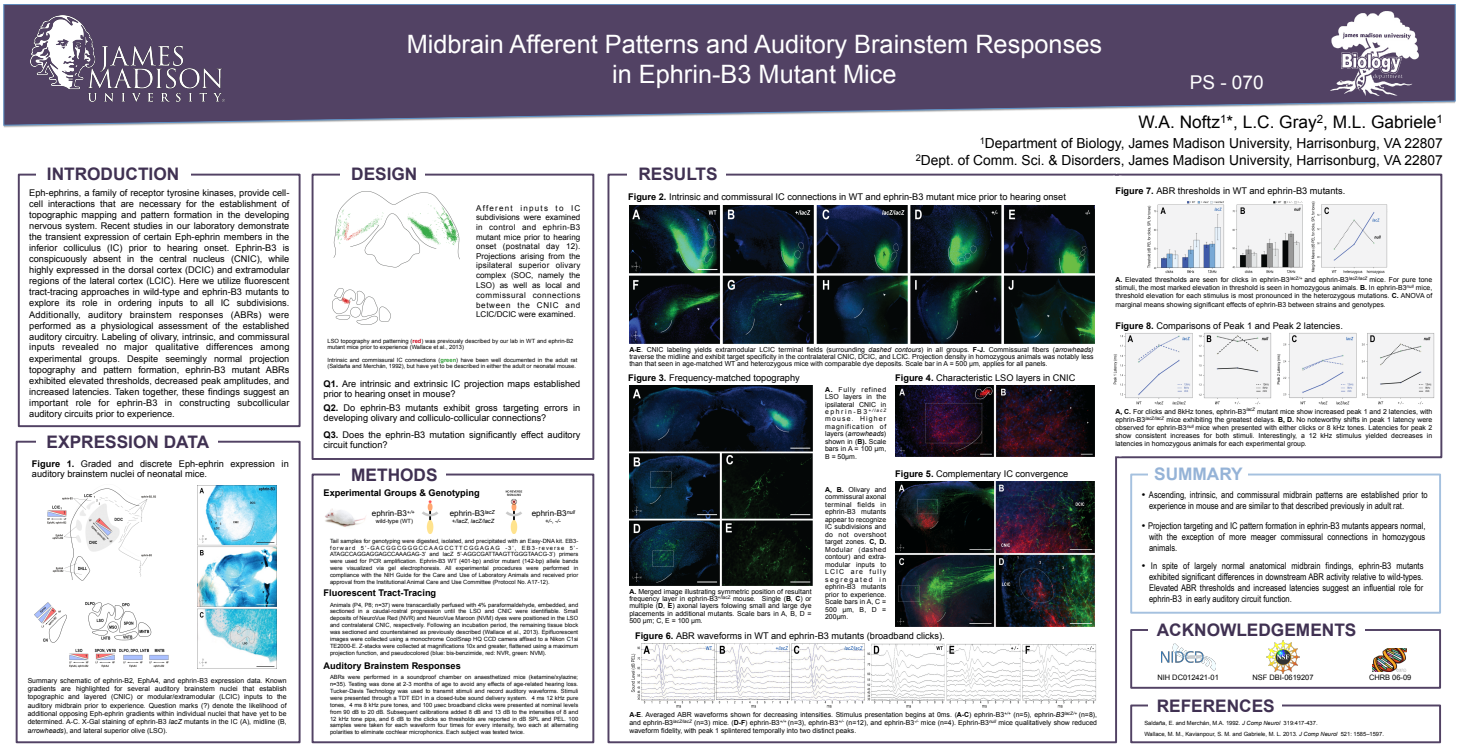

Figure 6. ARO poster for *Midbrain Afferent Patterns and Auditory Brainstem Responses in Ephrin-B3 Mutant Mice*

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